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BIOASSAY OF CHEMICALS WHICH LEAD TO RESISTANCE TO FLEA BEETLES AND OTHER CANOLA INSECT PEST IN CRUICIFER GERMPLASM

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Bioassays of Chemicals which Lead to Resistance to Flea Beetles and other Canola Insect Pests in Crucifer Germplasm

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Abstract

This project investigated the responses of flea beetles, cabbage root maggot adults and larvae, and bertha armyworms to commercially obtained phytochemicals, leaf extracts of selected crucifer species, and/or intact crucifer seedlings and plants. Efforts concentrated on identification of the phytochemical compounds to which insects responded negatively. Promising chemicals were then tested in serial dilutions to determine at what minimum level a bioactive response was invoked. The research provided details of the biochemical nature of host plant selection by these crucifer pests, a critical step in our strategy to develop insect resistant canola. The larval development of cabbage root maggot and bertha armyworm was monitored when a variety of phytochemicals, leaf extracts, and plant materials was added to their diet. Evidence of decreased larval ability to survive and develop was found, indicating the possibilities of developing canola foliage that is unhealthy for pest insects.

In the process of conducting the bioassays, methodologies were developed that will allow for rapid field screening of large numbers of seedling plants against flea beetle feeding. Progress has been made in maintaining test colonies of flea beetles over long periods of time. Further, the development of a meridic diet holds promise for rearing and testing the feeding preferences root maggot larvae.

Executive Summary

The goal of this project was to conduct bioassays on insect pests of canola production, testing their response to crucifer germplasm and associated plant chemical compounds. The ultimate aim of the research is to develop canola that is resistant to the pests, while making sure that imparting resistance to one pest does not invoke susceptibility to another.

The type of bioassay employed was dependent on the insect under investigation. For flea beetles, leaf disk bioassays were the principal test type. In this test, leaf disks of a susceptible plant, *Brassica napus* AC Excel, were treated with test chemicals and the subsequent insect feeding level monitored. The leaf disk tests were of two types - choice or no choice. In the choice test, beetles were placed in petri dishes with pairs of leaf disk treatments, AC Excel treated with solvent and test phytochemical, or with solvent alone; in the no choice test beetles were placed in dishes with only one treatment, and their feeding compared to feeding on the other treatment.

Because of the diverse chemical properties of the phytochemicals and plant extracts being tested, different solvents were necessary to suspend the chemical in solution for placement on the leaf disks. Different solvents dissolved different fractions of the chemicals. By using an array of solvents, inference could be made as to the nature of the chemical moiety to which the beetles most strongly responded.

Previous research showed that the crucifer species *Crambe abyssinica, Camelina sativa,* and *Thlaspi arvense* are all resistant to flea beetle feeding to some degree. The chemical profiles of these species were examined and phytochemicals present in them that were not present in susceptible species were tested for flea beetle response. Several phytochemicals, in particular flavone and some of its derivatives, consistently deterred flea beetles from feeding.

Numerous lines of *Arabidopsis thaliana* with known genetic mutations were tested for their resistance or susceptibility to flea beetle feeding. This was done in the expectation of

gathering information on the genetic basis of host plant resistance to flea beetles. Two groups of mutants from stock sources, entries with altered glucosinolate profiles and with the Gelbherzig characteristic of yellow first leaves, were resistant to flea beetle feeding in the laboratory and in the field.

Root maggot females were tested for their choice of host plant for laying eggs. In comparisons between AC Excel and yellow mustard *Sinapis alba* lines with varying glucosinolate and erucic acid contents, the flies consistently chose to lay their eggs beside the *Sinapis* plants. This selection did not appear to be linked to basal stem diameter.

However, when root maggot eggs were placed near the two plant species in choice tests, the resulting larvae developed normally on AC Excel, while avoiding *S. alba*. In no choice tests, newly emerged larvae initially fed on *S. alba*, but larval development decreased with time as mortality increased. Pupal development on yellow mustard was greatly retarded.

Leaf disk and whole plant bioassays were undertaken to test bertha armyworm larval responses to phytochemicals and leaf extracts. As with flea beetle feeding, flavone again proved a deterrent. As well, this chemical had detrimental effects on armyworm growth and development. Typical of a generalist feeder as opposed to flea beetles, which are crucifer specialists, bertha armyworms did not have strong feeding preferences among *Brassica napus*, *Crambe abyssinica*, or *Camelina sativa* leaf disks, and larval development on all three plant species was relatively normal.

In addition to numerous bioassays testing insect response to phytochemicals, leaf extracts and whole plants, project activities involved developing the protocols for conducting some of these bioassays. A bioassay method was developed that will allow for rapid field screening of *Arabidopsis thaliana* lines, of which 50, 000 activation-tagged entries produced in-house await assessment. Cold storage of flea beetles allows for the extension of laboratory bioassays into the winter months. And development of a meridic diet for root maggot larvae will allow for the accurate assay of phytochemicals that negatively affect them.

The activities of this project have greatly added to the knowledge of the biochemical nature of host plant selection by insects, and have increased insight into cues that retard proper insect growth and development. Accumulation of this information brings the goal of canola resistance one step closer to fruition.

Technical Report

The Technical Report detailing the activities of the project is presented on pages 7 to 45.

Personnel

A staffing competition was held and an entomological technician, J. M. Lazorko, was hired for a three-year term at an EG-ESS-01 level. A chemist, Dr. Joseph Onyilagha, was contracted to extract phytochemical constituents of *Camelina sativa* and *Sinapis alba* for use in flea beetle bioassays. Two summer student assistants were hired in the final year of the project, and casual labour was used for brief periods when extra technical assistance was required.

Equipment

No equipment was purchased to undertake this project.

Project Development Materials

a) scientific publications:

Onyilagha, J., Bala, A., Hallett, R., Gruber, M., Soroka, J., Westcott, N. 2002. Leaf flavonoids of the cruciferous species, *Camelina sativa*. (L.) Crantz, *Crambe* spp., *Thlaspi arvense* and several other genera of the family Brassicaceae. Submitted to Biochemical Systematics and Ecology.

Onyilagha, J.C., Gruber, M.Y., Soroka, J.J., Erlandson, M.A. and Lazorko, J. 2002. Effect of flavonoids on feeding behavior of the crucifer pest *Mamestra configurata* Walker (Lepidoptera: Noctuidae). Submitted to J. Chemical Ecology.

Hallett, R.H., Ray, H., Gruber, M.Y., and Soroka, J.J. Assessing crucifer flea beetle resistance in *Arabidopsis thaliana*. In preparation for Journal of Chemical Ecology.

Soroka, J.J., Gruber, M.Y., and Lazorko, J.M. Resistance and susceptibility of crucifers to cabbage root maggot (*Delia radicum* L.) in the laboratory. In preparation for The Canadian Entomologist.

b) technology transfer productions:

Gruber, M., Hegedus, D., Xu, N., Yu, M., Baldwin, D., Lazorko, J., Sharpe, A., Westcott, N., Soroka, J., Parkin, I. 2002. Resistance to flea beetles. Oral presentation by M. Gruber. In: Abstracts. Genomics. AAFC Canadian Crops Genomics Initiative Annual meeting. London, ON. June 13-14, 2001.

Xu, N., Lazorko, J., Gruber, M., Westcott, N., Soroka, J. and Hegedus, D. 2002. Metabolic profiling of *Arabidopsis* volatiles that modify flea beetle electrophysiological behavior. In: Abstracts. Plant Biology Canada 2002. Annual meeting. Canadian Society for Plant Physiology. Calgary Alberta. June 8-12. p. 64.

Soroka, Julie, Larry Grenkow, Richard Gugel, and Margaret Gruber. 2001. Resistance of *Crambe* and *Camelina* to insect pests. Oral presentation at Entomological Society of Canada, Joint Annual Meeting, Niagara Falls, ON, October 21 - 24, 2001 **and** poster presentation at Entomological Society of America, San Diego, CA, Dec. 9 - 2, 2001.

Gruber, M., Lazorko, J. and Soroka, J. 2001. Crucifer flea beetles, *Phyllotreta cruciferae*, and induced mutations in the model plant, *Arabidopsis thaliana*. Poster presentation at Entomological Society of America, San Diego, CA, Dec. 9 - 2, 2001.

Soroka, JJ and R. Rugel. 2001. No Bugs on *Camelina* - a candidate for Molecular Farming? Oral presentation at Western Committee on Crop Pests annual meeting, Banff, AB, Oct 14 - 16, 2001.

Xu, N., Lazorko, J., Gruber, M., Westcott, N., Soroka, J., Parkin, I., and Hegedus, D. 2001. Linking metabolic profiles with bioactivity to identify *Arabidopsis* genes that influence crucifer insect behavior. Poster presented at Phytochemical Society of North American Annual Meeting. Oklahoma City, OK. Aug 4-8, 2001. In: Abstracts: Phytochemistry in the genomics and post-genomics eras. Annual Meeting. Phytochemical Society of North America. Oklahoma City, OK. Aug 4-8. Poster PI-36. p. 50.

Gruber, M., Hegedus, D., Westcott, N., Soroka, J., 2001. Resistance to flea beetles. Oral presentation by D. Hegedus. In: Abstracts. Canadian Crop Genomics Annual meeting. Winnipeg. June 5-6, 2001.

Gruber, M., Hegedus, D., Westcott, N., Soroka, J., 2001. Resistance to flea beetles. Oral Presentation by M. Gruber. 1st Annual PBI-NRC/ SRC Genomics Meeting, Saskatoon, July 18-19, 2001.

Project Photographs

Relevant photographs are included as Photographs 1 to 4 on pages 40 to 42.

Acknowledgments

Support by the Saskatchewan Department of Agriculture and Food Agricultural Development Fund was acknowledged for all technological transfer presentations and posters, and will be acknowledged in all future publications and presentations arising from the project.

We would like to thank Larry Grenkow, AAFC Saskatoon Research Centre, for his able technical and statistical assistance in conducting this project.

BIOASSAYS OF CHEMICALS WHICH LEAD TO RESISTANCE TO FLEA BEETLES AND OTHER CANOLA INSECT PESTS IN CRUCIFER GERMPLASM

FINAL REPORT, SEPTEMBER, 2002
Dr. Juliana J. Soroka

TECHNICAL REPORT

Objective:

The objective of this project was to conduct insect pest bioassays on crucifer germplasm and associated phytochemicals with the ultimate goal of developing insect resistant canola.

Project Activities:

The activities of this project centered around three canola insect pests: flea beetles (*Phyllotreta* spp., principally *Phyllotreta cruciferae* Goeze in the field; *P. cruciferae* in the laboratory); crucifer-feeding root maggots *Delia radicum* L., in the laboratory, and bertha armyworm, *Mamestra configurata* Walker, in the laboratory.

Material tested included plant extracts and commercially obtained phytochemicals placed on *Brassica napus* leaf disks in choice and no-choice feeding bioassays. Intact seedlings and plants of several crucifer species, including wild-type and mutagenized *Arabidopsis thaliana* L., lines, were also tested for feeding susceptibility or resistance. Resistant plant species tested included crambe, *Crambe abyssinica* Hochst, false flax *Camelina sativa* (L.) Crantz, and stinkweed *Thlaspi arvense* L. As well as feeding tests, oviposition (egg-laying) trials were conducted testing responses of female root maggots towards several plant species.

1. Flea Beetle Bioassays:

Laboratory leaf disk bioassays of flea beetle feeding constituted the majority of experiments conducted in this segment of the project. Commercially obtained phytochemicals or plant tissues were homogenized, dissolved in a series of solvents, and eluted onto disks of *Brassica napus* cultivar AC Excel leaf placed on agar in plastic arenas. The level of feeding on the leaf disks was determined after substantial feeding on the control disk had occurred, usually after 24 or 48 hours. In choice tests, flea beetles were allowed to feed on treated or untreated *B. napus* leaf disks in the arena. In no choice tests, flea beetles were given only one material upon which to feed. Serial dilutions of phytochemical extracts were undertaken to determine the level of chemical at which a biological response by flea beetles occurred. The complete methodology for leaf disk bioassays is outlined in the Appendix on pages 37 to 39.

a. *Crambe abyssinica* leaf extracts in various solvents:

Crambe abyssinica is a commercially produced crucifer with resistance to flea beetle feeding (Anderson et al. 1992). In a leaf disk bioassay of *Crambe abyssinica* leaf extracts using four different solvents to dissolve different phytochemicals, of greatest interest were those leaf

extracts with a feeding deterrence index value of greater than 25, for these have the greatest potential as sources of resistance to flea beetles. The results indicated that the leaf phytochemical components most active against flea beetles were not soluble in distilled water, but were present in the methanol, hexane, and, to a lesser degree, dichloromethane fractions (Table 1).

Repetitions of the bioassay affirmed the deterrence of the leaf extracts dissolved in dichloromethane and methanol to flea beetle feeding (Figure 1).

b. *Thlaspi arvense* leaf extracts in different solvents:

Leaf disk bioassays similar to those with *Crambe* were conducted testing *Thlaspi arvense* leaf extracts. *T. arvense*, or stinkweed, also shows resistance to flea beetle feeding (Palaniswamy et al. 1997). Results of the bioassay are presented in Table 2.

Leaf disks treated with the dichloromethane and methanol fractions of the leaf extractions in both plant species had the greatest feeding deterrence to flea beetles. Components of the hexane fraction of *C. abyssinica* leaf extracts appeared to be more deterrent than the hexane fractions of *T. arvense* leaf extracts. In general, chemical constituents of *Crambe* leaves appeared to be stronger deterrents to flea beetle feeding than those of *Thlaspi*.

Further leaf disk bioassays conducted with *C. abyssinica* and *T. arvense* leaf extracts, including repeated testing and serial dilutions of extracts, showed similar results.

c. Combined *C. abyssinica, T. arvense*, and *Camelina sativa* Bioassay:

In the fall of 2000, a bioassay was conducted that simultaneously tested three concentrations of each of *C. abyssinica, T. arvense*, and *C. sativa* leaf extracts, dissolved in one of four or five different solvents, for a total of 39 treatments plus controls, with 15 replicates of each treatment. Generally, leaf extracts of all three crucifer species deterred flea beetle feeding compared to leaf disks treated with solvent alone. The solvents that extracted the most active leaf extract fractions included hexane, dichloromethane, and methanol (Figures 2 to 4). Generally, feeding was deterred most when the greatest concentration of extract, 10 mg/ml, was used (e.g. Figure 2). However, leaf extracts of *Crambe* extracted with hexane and tested in a low concentration of 1 mg/ml had substantial levels of feeding deterrence (Figure 2). Low concentrations of acetonitrile (ACN) (Figure 4) and butanol (Figure 5) subfractions of *Camelina* leaf extractions also provided high levels of feeding deterrence.

In order to further elucidate the nature of the phytochemicals affecting flea beetle feeding, leaf extracts of *C. abyssinica, T. arvense*, and *C. sativa* were developed using a sequential series of non-polar and polar solvents. Polar extracts were separated on a C18 column using high pressure liquid chromatography (HPLC) and specific sub-fractions were eluted, concentrated and tested in flea beetle bioassays. Non-polar extracts were separated by gas chromatography and the components identified by mass spectroscopy. Several of the polar subfractions in all three crucifer species were found to have very high feeding deterrence (Figures 6 and 7). In particular, chlorogenic acid fractions in *Camelina* and flavone fractions in *Crambe* and *Thlaspi* were deterrent to flea beetle feeding. Non-polar fractions were also deterrent.

e. Bioassays of *Camelina sativa* leaf extracts fractioned with Acetonitrile:

Bioassays were conducted to test the activity of HPCL-fractionated *C. sativa* leaf components against flea beetle feeding, using 15% acetonitrile as the fractionation carrier. This 15% ACN fraction was previously found to be highly deterrent to flea beetles at a concentration of 5 mg/ml leaf extract in methanol solvent. It was further fractionated into 17 component compounds. The ten sub-fractions that were collected in the greatest quantity were tested at concentrations of 1.0 and 0.2 mg/ml with variable results (Table 3). The higher concentration of the fractions had greater feeding deterrence than did the 0.2 mg/ml concentration. There were some similarities in the rankings of the fractions at the two concentrations; the fraction with the second highest deterrence value at the high concentration had the highest deterrence at 0.2 mg/ml, and the fraction with the least deterrence at 1.0 mg/ml had the second lowest deterrence at the low concentration (Table 3).

f. Trichome Density Bioassay:

Bioassays were conducted to test the effect of trichome density as expressed in transgenic Westar lines of *B. napus* on flea beetle feeding. Trichomes, or stiff plant hairs, impart resistance to a variety of insects in many plants, including yellow mustard *Sinapis alba* L., pod resistance to flea beetles (Lamb 1980). In five experiments, individual comparisons between 15 different transgenic lines and Westar control plants were made, with up to six replications of entries. Many of the 15 lines exhibited reduced feeding over the Westar controls, as exemplified in Figure 8. This figure summarizes an experiment in which, among others, the transgenic line 152-D8-2 had a feeding deterrence index of 48%. Agronomic improvement of enhanced-trichome lines of Westar continues.

g. Bioassay of *B. napus* Lines with Varying Levels of Anthocyanins:

One of the characteristics of some of the mutant Westar lines with increased trichome levels (see f above) was elevated quantities of anthocyanins, visible as increased purple colour in the leaves. Anthocyanins are antioxidant flavonoids that provide pigment for pansies, petunias, and the fall colours of deciduous forests. A *B. napus* bioassay was conducted and repeated to test the effect of anthocyanin concentration on flea beetle feeding. Six entries with varying levels of anthocyanin were tested, two of which had feeding levels consistently below those of the Westar control.

h. Flea Beetle Bioassays Assessing *Arabidopsis thaliana* Mutant Lines

I) Considerable time was spent in bioassay of flea beetle feeding on *A. thaliana* mutant lines, in the hopes of gaining insight into the genetic basis of plant resistance or susceptibility to flea beetles. The genome or genetic code of *A. thaliana* has been determined, and this small crucifer is being used as an indicator plant for crucifer molecular genetic programs.

Results of previous laboratory bioassays (Photograph 3 G) of *Arabidopsis* mutant lines obtained from the *Arabidopsis* Biological Resource Centre, Columbus, Ohio, were re-evaluated using a new SAS statistics program that compared experiments done on different days, phenology of flea beetle (spring or fall populations), feeding preference based on cotyledons, feeding preference based on true leaves, and feeding preference on the whole seedling. Results

were homogeneous enough to allow the summarization of the data into tables of feeding deterrence/susceptibility. The results showed that several of the mutant lines have feeding levels lower than the control *A. thaliana* cv Columbia. These lines were investigated further (cf Figure 9).

ii) A preliminary protocol for the large-scale testing of *Arabidopsis* mutant lines in the field was developed. The object of the experiment was to test the mutants against flea beetles in the field so as to compare field results with those previously obtained in the laboratory, and to develop a methodology for rapid screening of large numbers of *A. thaliana* lines.

In preparation for the field test, a trial was conducted to determine the best method of cold treating *Arabidopsis* seed in order to get even germination on soil-less mix. Two trials were conducted to determine minimal pot size and orientation required to attract flea beetles for field bioassay of *Arabidopsis* mutant lines (Photograph 3 D and F). From these tests a methodology was developed that allows for concurrent field testing of up to 50 lines of *A. thaliana* (Photo 3 E).

iii) Two bioassays evaluating *Arabidopsis* mutant lines were conducted in September, 2001, the first in early September when flea beetle feeding pressure was very high. Eight of the 11 lines evaluated in the first field bioassay had susceptibility/resistance rankings similar to those found in previous laboratory bioassays. The most susceptible mutants in the field were two lines with altered accumulation of anthocyanins in the cotyledons, while the four most resistant mutants were two lines with altered glucosinolate metabolism (entries 2226 and 2227, Figure 9), and two lines with the Gelbherzig characteristic of yellow leaves (entries 3297 and 3288, Figure 9). In the second bioassay, feeding levels generally were low and not as distinct among entries, but the most and least susceptible lines were similar to those of the laboratory and first field bioassays.

In 2002, 21 separate field bioassays of mutant *A. thaliana* lines were attempted, with dismal results. Perverse weather conditions in both spring and summer meant that at no time did ideal plant growth, heavy flea beetle populations, and suitable feeding weather coincide. Five trials had feeding levels that were high enough to be rated. Some trends in feeding resistance/susceptibility could be discerned. Flea beetle numbers and feeding levels increased dramatically with the onset of the summer generation of flea beetles in September. Bioassays in early and mid-September had sufficient feeding to discern resistant and susceptible *Arabidopsis* lines. The next step is to test unique *A. thaliana* lines developed at Saskatoon Research Centre.

2. Flea Beetle Collection, Population Monitoring, and Feeding Damage Rating Standardization:

a. Flea Beetle Collection:

Because no method has been successfully developed for maintaining a flea beetle colony year-round in the laboratory, it was necessary to collect wild flea beetles from the field throughout each growing season for use in bioassays. Overwintering flea beetles were collected starting in early May by sweeping with a standard 38 cm diameter insect sweep net, at first from local patches of flixweed, *Descurainia sophia* (L.) Webb., and then later from seedling canola; or in modified yellow boll weevil traps baited with allyl isothiocyanate (mustard oil) (Photograph 1 left), a flea beetle attractant. The traps were placed in tree shelterbelts surrounding canola stubble fields in early spring, then moved to the borders of canola plots once the seedlings

had germinated. Flea beetles were collected from the field until early October, whereupon freezing temperatures and snow forced them to cease feeding and seek overwintering sites. Collected flea beetles were returned to the laboratory, placed in large plexiglass maintenance cages, and fed cabbage and distilled water so as to provide a uniform pool of test insects. Some success was achieved in placing flea beetles in cold storage at 2°C, to be used in laboratory feeding bioassays later in the fall and winter. Flea beetle mortality increased with increased length in cold storage; however, when removed from 2°C, surviving flea beetles fed relatively normally. Thus, bioassays were able to be conducted until well into February, and were terminated only when the supply of surviving beetles decreased below the number required to conduct replicated trials.

b. Flea Beetle Population Monitoring:

Sweep net and baited boll weevil trap monitoring of flea beetles was conducted throughout each season of the project. However, the number of flea beetles collected by both these methods tapered off in mid-summer as the previous generation of adults died off and the new generation had yet to emerge. Emergence cone traps, circular cones of approximately 40 cm in diameter placed over the soil near canola plants (Photograph 1 right), were placed in winter canola plots at the Saskatoon Research Centre farm in July to determine the size and phenology of the summer generation of flea beetles. In 2001 peak emergence of the summer generation flea beetles occurred around August 31, 1 week earlier than the population peak in 2000 (Figure 10). In 2002, peak summer generation emergence was delayed beyond the date of the writing of this report (early September). The size of the curves in 2000 and 2001 indicated the potential for high populations of flea beetles in the following year, which, indeed, did occur.

c. Flea Beetle Feeding Damage Rating Standardization:

Concern has been raised by canola pest management specialists that the flea beetle economic threshold, obtained by a visual assessment of percent area eaten of canola cotyledons and first true leaves, is subject to variability depending on the assessor's ability to accurately estimate the degree of feeding scars. To address this concern, and to validate the methodology used in our bioassays, a feeding rating scale was constructed, utilizing cotyledons that had been fed upon by flea beetles to varying degrees. The feeding damage to these cotyledons was first estimated 'by eye', and then measured with a digital image analyzing system (DIAS). Similar results were obtained by both methods, but visual assessment was much more rapid than was DIAS measurement. The rating scale is presented in Photograph 2.

3. Root Maggot Bioassays:

a. Root maggot Oviposition on *Sinapis alba* Lines

For larvae that have limited mobility, it is the choice of host for oviposition sites of the female adult insect that largely determines on what plant the larvae feed. Therefore, several plant species were tested in the laboratory for their attraction or deterrence to egg-laying root maggots.

Choice and no-choice tests were set up in which 10 newly emerged, colony-reared root maggot adults, eight females and two males, were placed in each of 10 cages containing either or both of two test plants. Four bioassays in total were conducted. In Trial 1 the plants tested were a low erucic acid, low glucosinolate line of *Sinapis alba*. In Trial 2, *S. alba* cv. Ochre plants with normal erucic acid and normal glucosinolate levels were tested. In Trial 3, we examined the oviposition preference of maggot flies to a high erucic acid, low glucosinolate line of *S. alba*. In Trial 4 a line of *S alba* with no erucic acid and normal glucosinolates levels was tested. In all three trials *Brassica napus* cv. AC Excel plants served as the control.

When flies were given a choice, there was a marked avoidance of AC Excel plants as egg hosts and a strong preference for laying eggs at the base of *S. alba* plants, no matter what their erucic acid and glucosinolate profile (Table 4). From the data in Table 4, it appears that level of erucic acid and glucosinolates do not influence egg laying of root maggot females. Number of eggs laid by cabbage maggot has been previously found to be correlated with large basal stem diameter (Dosdall et al. 1995, 1996). This was not the case in the current study, in which most AC Excel plants had larger root crowns than did *S. alba* plants.

In the no choice tests, female flies may have been reluctant to lay their eggs on AC Excel plants with *Sinapis* plants in nearby cages, for in all four trials the number of eggs laid on *S. alba* plants exceeded the number on AC Excel plants (Table 5). However, the physical necessity of laying eggs somewhere forced egg laying on AC Excel crowns, and in two of the four trials numbers of eggs laid were statistically similar on both crucifer species.

b. Bioassay of Root Maggots Feeding on *B. napus* lines with Increased Levels of Protease Inhibitors:

One bioassay was conducted and repeated, testing seven lines of *B. napus* cultivar Westar mutants containing varying levels of protease inhibitors for evidence of root maggot resistance. Thirty root maggot eggs collected from the colony were placed at the base of each of 10 test plant entries, ten replicates of which were then maintained in the greenhouse (Trial 1) or growth chamber (Trial 2) under standard conditions for 30 days. The subsequent root maggot damage level, on a scale of 0, undamaged, to 5, root completely eaten or severed (Dosdall et al. 1994), and number of root maggot larvae and pupae surviving on plants of each of the test lines were determined. No differences were found in the amount of feeding by maggots on any of the 10 entries tested, or in the number of larvae found per entry (Table 6). Further, all of the elevated protease inhibitor lines supported greater numbers of root maggot pupae than did the standard AC Excel (Table 6), indicating that this trait did not negatively affect the biology of the maggots. Interestingly, white mustard *S. alba* supported the lowest number of pupae (Table 6).

c. Comparison of Root Maggot Larval Feeding and Development on *B. napus* and *S. alba*:

A no-choice bioassay was conducted to test root maggot larval feeding and development on one of two different test plant species for three different time periods. Maggots emerging from eggs that had been placed near *B. napus* AC Excel plants developed normally and caused typical feeding damage to roots for the length of their developmental period. There was initial feeding on *S. alba* roots by newly emerged *D. radicum* larvae, but as the test progressed fewer maggots survived, and no larvae developed to pupation (Table 7). The results indicate that the *S. alba* roots contain factors that are antibiotic and/or antixenotic factors to the maggots.

A second root maggot bioassay was conducted in which a choice between *B. napus* AC Excel and *S. alba* Ochre was offered to 30 root maggot eggs per pot. A greater number of larvae was found on AC Excel roots than on Ochre roots (Table 8). As well, AC Excel had more root feeding than did Ochre, and larvae found on AC Excel roots weighed more than those found on Ochre, attesting to the anti-nutrient quality of the mustard roots.

d. Bioassay of Root Maggots Fed on Crucifer Varieties:

In order to further investigate the interactions between root maggots and host plants, a bioassay was undertaken in which 20 root maggot eggs were placed near plants of five species of crucifers growing singly in pots in the greenhouse in 10 replications. After pupation, plants were cut down to soil level, and the plant height, wet and dry weights of plants infested and uninfested with maggots were measured. The root damage levels of plants infested with maggots, the number of maggots reaching pupation, and the average weight of pupae were also recorded.

At the damage levels seen in this experiment, no differences in plant growth attributes were found between infested and uninfested plants (data not presented). Neither were there any differences in feeding damage among the six entries of the trial (Table 9). There was a trend for decreased number of pupae and pupal weight in pots of *S. alba* and *B. carinata* (Table 9).

e. Root Maggot Diet Development:

To date, little success has been achieved in utilizing the one commercial artificial diet (Bio-Serv, Inc.) available for cabbage root maggot rearing. On this diet root maggot eggs have hatched and larvae have survived until reaching the second instar only. In our laboratory root maggots are routinely reared on rutabaga root embedded in sterile sand. If normal growth and development of maggot larvae could be obtained on a meridic diet (one which consists of mostly defined chemicals but which includes undefined components such as rutabaga root), we would be able to test candidate phytochemicals by adding them, in serial concentrations, to the diet and monitoring larval development and behaviour. To this end an artificial diet for *Delia* larval growth is being developed. The commercial root maggot diet contains yeast, sucrose, ascorbic acid, methyl paraben, choline chloride, pea flour, salt mix and corn oil in unknown quantities, as well as agar to maintain a gel-like consistency. In 20 bioassays, rutabaga root in varying proportions was added to the commercial diet mixture and the development of known numbers of eggs per diet cup was monitored over a one month period for each bioassay. Rutabaga root in

several forms - ground rutabaga slurries, rutabaga water extracts, and dried, ground rutabaga roots - was added to the diet and evaluated. Incorporating rutabaga appeared to increase the longevity of larvae on the diet; in several bioassays normal-appearing pupae developed. However, the greater the amount of rutabaga added, the greater the contamination of the diet by fungi, slimes, and molds, which were detrimental to larval health. The diet is in the process of being refined to maximize pupal development while minimizing pathogen development.

4. Bertha Armyworm Bioassays:

Results from experiments on bertha armyworm feeding preferences varied, attesting to the polyphagous nature of the insect.

a. Bioassay of Bertha Armyworm Feeding on Flavones:

I) Feeding bioassays testing flavone and flavonoid compounds against bertha armyworm larvae were performed. A range of feeding response was found, with most compounds having little pronounced effect on feeding (Figure 11). However, flavone and 4-hydroxyflavone produced 25% feeding deterrence or greater.

ii) To investigate the usefulness of the apparent deterrence of flavone to bertha armyworm, a bertha feeding bioassay testing serial dilutions of flavone was preformed. The results reaffirmed the consistency of previous feeding deterrence data. This naturally occurring phytochemical showed deterrence to flea beetle feeding at fairly low concentrations (Figure 12). Fifty percent feeding deterrence was achieved at 0.12% fresh weight of leaf material.

iii) Feeding bioassays were conducted measuring the effects of three concentrations of flavone in bertha armyworm diet on larval growth and development. Early third instar larvae were placed on one of three diets, regular diet, regular diet with water and ethanol solvent, and regular diet with water, ethanol, and flavone at one of three concentrations, 100, 150, and 200 µg per gram of diet. Larval development was monitored and weights were measured every two days. At the lowest concentration of flavone in the diet, no differences were seen in time of development to pupation, mean relative growth rate, larval weight gain or mortality among any of the three diets (data not shown). At 150 µg per gram diet, lower relative growth rates, rates of development to pupation, and larval weights were found in larvae that had consumed flavone, as compared to those that had not (Figures 13 and 14). At 200 g. per gram of diet, relative growth rate, time to pupation, and weight of larvae feeding on flavone were strongly depressed, while rate of mortality was greater than in larvae not feeding on flavone (Figures 13 and 14).

b. Bioassay of Bertha Armyworm Feeding on Three Crucifer Species:

A bertha armyworm bioassay was conducted and repeated testing the insects' feeding preference on three host plants in leaf disk binary choice bioassays and in whole plant no-choice bioassays at two different plant growth stages. The plant hosts tested were *Brassica napus* cv AC Excel, *Crambe abyssinica* JS00-819, and *Camelina sativa* JS00-1004 at the six leaf (approximately 24 day) stage, and at pre-bolting (approximately 42 days after emergence); early

second instar bertha armyworm larvae were used in the tests. Three larvae were tested in each choice test plate (Photograph 4), while six larvae were placed on each no-choice whole plant. Choice test leaf disks were rated for feeding damage 18 hours after test initiation. In the choice tests, there was reduced feeding on *C. sativa* when placed with either *B. napus* or *Cr. abyssinica* at the six leaf stage (Table 10). This also held true at the pre-bolting stage for *B. Napus* - *C. sativa* pairs, but not for *Cr. abyssinica* - *C. sativa* tests. Little difference was seen in feeding levels between *B. napus* or *Cr. abyssinica* when bertha armyworm larvae were given a choice of leaf disks from these two plants (Table 10). In the whole plant no-choice trial of this experiment, the level of feeding at both the six leaf and pre-bolting stage was similar among plant species. Feeding on *Cr. abyssinica* was more variable in quantity than feeding on the other two species. No significant differences were found in larval weights after feeding. Thus it appears that, while bertha armyworm larvae express some feeding preferences to the crucifer species tested, they can feed and develop equally well on all three.

5. Project Summarization:

The preparation of a Feeding Deterrence Index summary for all the commercial compounds tested against flea beetles, bertha armyworm, and diamondback moths from the past four years was undertaken (addendum, pages 43 to 45). This allowed for an overall assessment of the impact of commercially available phytochemicals on insect feeding.

Table 1. Leaf disk bioassay determining feeding deterrence index of *Crambe abyssinica* leaf extracts mixed with one of four solvents tested against flea beetles in the laboratory. Feeding deterrence index was calculated by subtracting average leaf area eaten on disks with *C. abyssinica* leaf extracts plus solvent from control feeding levels (leaf disks treated with solvent alone), dividing by leaf area eaten on both types, and multiplying by 100%.

Crambe leaf extract plus	Feeding Deterrence Index*
water	-6.7
hexane	85.2
dichloromethane	67.6
methanol	98.8
control (no solvent)	15.0

*the greater the FDI value, the greater the deterrence. Negative values represent feeding stimulants.

Table 2. Leaf disk bioassay determining feeding deterrence index of *Thlaspi arvense* leaf extracts mixed with one of four solvents tested against flea beetles in the laboratory. Feeding deterrence index was calculated by subtracting average leaf area eaten on disks with *T. arvense* leaf extracts plus solvent from control feeding levels (leaf disks treated with solvent alone), dividing by leaf area eaten on both types, and multiplying by 100%.

Thlaspi leaf extract plus	Feeding Deterrence Index*
water	15.2
hexane - 10 mg/mL	35.3
hexane - 1mg/mL	0.8
dichloromethane	72.2
methanol	52.4
control (no solvent)	16.0

*the greater the FDI value, the greater the deterrence. Negative values represent feeding stimulants.

Table 3. Leaf disk bioassay determining feeding deterrence index of *Camelina sativa* leaf extracts derived from 15% acetonitrile high pressure liquid chromatography extraction and tested against flea beetles in the laboratory at two extract concentrations.

	1.0 mg/ml concentration of extract		0.2 mg/ml concentration of extract	
C. sativa fraction	Feeding Deterrence Index*	Standard Error	Feeding Deterrence Index	Standard Error
3	75.1	8.5	13.1	21.7
11	58.8	16.1	33.3	16.6
6	48	19.3	3.4	17.2
15	44.4	18.6	24.3	21.2
10	33.3	15.9	19.4	16.6
1	30.8	17.2	-49	16.5
2	22.2	19.4	6.7	22.1
4	21.2	21.8	1	21.6
8	20	24.3	25.7	18
13	-73.1	13.4	-44.4	19.2

* the greater the FDI value, the greater the deterrence. Negative values represent feeding stimulants.

Table 4. Number of eggs laid (and standard error of the mean) by cabbage maggot females at the base of plants of two species of crucifer when given a choice in three laboratory cage trials.

	Chemical Profile of *S. alba*	No. eggs laid on *S. alba*	No. eggs laid on AC Excel
Trial 1	↓erucic acid, ↓glucosinolates	253.5±51.3	23.2±10.0
Trial 2	normal erucic acid, normal glucosinolates	112.0±28.9	48.7±14.5
Trial 3	↑erucic acid, ↓glucosinolates	447.2±32.0	66.3±36.3
Trial 4	0 erucic acid, normal glucosinolates	336.2±61.5	34.5± 7.6

Table 5. Number of eggs laid (and standard error of the mean) by cabbage maggot females at the base of plants of two species of crucifer in a no-choice bioassay in three laboratory cage trials.

	Chemical Profile of *S. alba*	No. eggs laid on *S. alba*	No. eggs laid on AC Excel
Trial 1	↓erucic acid, ↓glucosinolates	98.2±21.1	84.2±30.1
Trial 2	normal erucic acid, normal glucosinolates	184.5±34.5	72.5±27.3
Trial 3	↑erucic acid, ↓glucosinolates	274.2±38.9	214.8±46.3
Trial 4	0 erucic acid, normal glucosinolates	198.5±82.5	59.5±27.5

Table 6. Damage level to roots by cabbage root maggot, *Delia radicum*, and number of root maggot larvae and pupae (± standard error of the mean) developing from eggs placed near the crowns of *B. napus* cv Westar lines with increased levels of protease inhibitors.

Entry Tested	Root Damage Level (0-5)	No. Larvae	No. Pupae
PI332	3.2± 0.2	1.0 ± 1.0	19.5 ± 1.6
PI330	3.3 ± 0.2	0.2 ± 0.1	17.4 ± 1.8
PI329	3.1 ± 0.1	0.6 ± 0.3	15.5 ± 2.5
PI328	3.0 ± 0.2	0.5 ± 0.2	14.4 ± 1.7
PI333	3.2 ± 0.2	0.3 ± 0.2	14.3 ± 1.6
Westar	2.9 ± 0.2	0.4 ± 0.2	13.6 ± 2.0
PI331	2.7 ± 0.2	0.3 ± 0.2	13.2 ± 2.6
PI334	3.3 ± 0.2	0.3 ± 0.2	12.9 ± 2.5
AC Excel	2.9 ± 0.2	0.2 ± 0.1	12.5 ± 2.4
Ochre	3.0 ± 0.2	0.0 ± 0.0	9.1 ± 2.0

Table 7. Level of feeding damage (on a scale of 0 to 5, least to most damaged) to crucifer roots and number of larvae or pupae surviving from 20 root maggot eggs placed per plant on two crucifer species grown in a no-choice test in a growth chamber for three lengths of time.

Feeding period	7 days		14 days		21 days	
Species Tested	Root damage rating (0-5)	No. Larvae	Root damage rating (0-5)	No. Larvae	Root damage rating (0-5)	No. Pupae
B. napus AC Excel	1.2	7.2	2.5	7	2.5	9
S. alba Ochre	0.8	3	0	2.8	1.5	0

Table 8. Average level of feeding damage (on a scale of 0 to 5, least to most damaged) to crucifer roots and number of larvae or pupae surviving from 30 root maggot eggs placed per pot in a choice of two crucifer species grown per pot in a growth chamber for two lengths of time.

Feeding period	7 days			21 days		
Species Tested	Root damage rating (0-5)	No. Larvae	Ave. Larval Weight	Root damage rating (0-5)	No. Larvae	Ave. Larval Weight
B. napus AC Excel	1	3.7	0.036	2	3.3	0.044
S. alba Ochre	0.3	1	0.002	0.7	2	0.036

Table 9. Average damage level to roots by cabbage root maggot, *Delia radicum*, number of root maggot pupae, and pupal weigths (± standard error of the mean) developing from eggs placed near the crowns of five species of crucifers, one with two different levels of glucosinolates, grown in the greenhouse.

Entry Tested	Root Damage Level (0-5)	No. Pupae	Pupal Weight (mg)
B. napus AC Excel	2.2±0.4	9.6±1.9	125±25.8
B. rapa AC Boreal	2.3±0.3	9.0±1.6	99.9±23.8
S. alba low glucosinolate	2.3±0.4	7.7±0.9	92.0±17.5
B. juncea Vulcan	2.3±0.2	8.7±1.0	88.0±13.1
S. alba Ochre	2.3±0.2	6.4±1.2	77.4±16.0
B. carinata Dodolla	2.7±0.1	4.7±1.0	44.5±8.9
P*≤	0.86	0.07	0.09

*None of the species was statistically different from the others at P≤0.05

Table 10. Percentage leaf area eaten (±standard error of the mean) by bertha armyworm, *Mamestra configurata*, in an 18 hour period when presented with a choice of leaf disks of pairs of three crucifers in laboratory bioassays. Crucifers were tested at three weeks of growth with about 6 leaves, and just prior to bolting.

a) Six Leaf Stage:

Choice between	% L.A.E.	Choice between	% L.A.E.	Choice between	% L.A.E.
Brassica napus	25.0±3.7	*Brassica napus*	16.0±2.7	*Cr. abyssinica*	27.0±9.0
Camelina sativa	18.7±0.0	*Crambe abyssinica*	21.7±9.6	*C. sativa*	11.5±5.2

b) Pre-Bolting Stage:

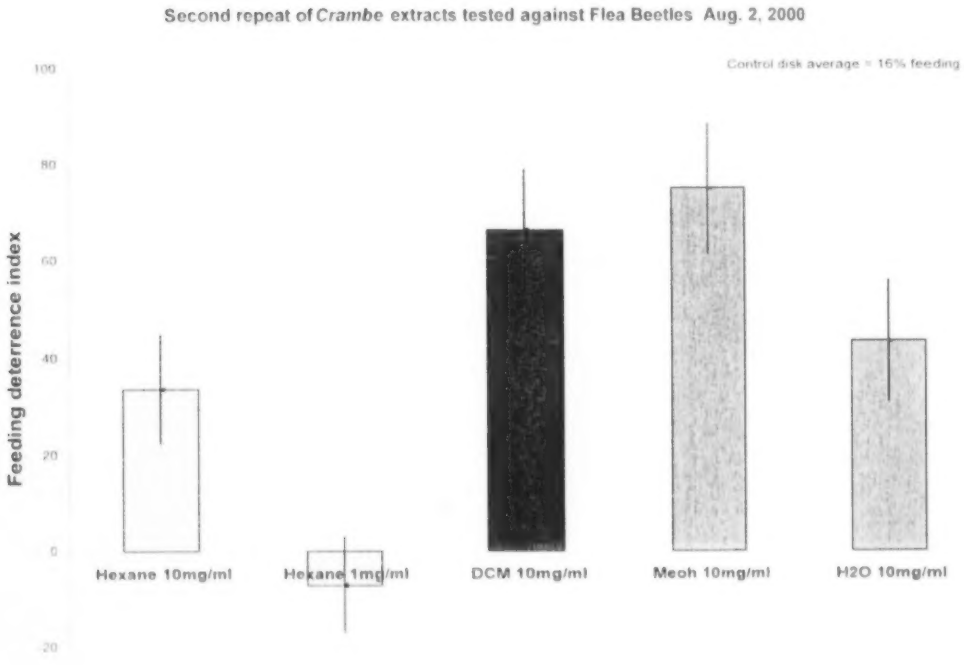
Choice between	% L.A.E.	Choice between	% L.A.E.	Choice between	% L.A.E.
Brassica napus	24.3±3.0	*Brassica napus*	14.4±1.6	*Cr. abyssinica*	8.4±7.2
Camelina sativa	11.6±4.5	*Crambe abyssinica*	8.0±7.4	*C. sativa*	15.4±0.3

Table 11. Feeding level and post-feeding larval weights (±standard error of the mean) of bertha armyworm, *Mamestra configurata*, feeding on whole plants of one of three crucifers in no choice laboratory bioassays. Crucifers were tested at three weeks of growth with about 6 leaves, and just prior to bolting.

a. Six Leaf Stage	Feeding Level[1]	Larval Weight Post-feeding (mg)
Brassica napus	30.3±3.0	3.0±0.0
Crambe abyssinica	38.8± 2.5	3.2±0.2
Camelina sativa	34.0± 8.0	2.8±0.2
b. Pre-Bolting		
Brassica napus	17.2± 0.5	3.5±0.4
Crambe abyssinica	26.2±20.2	3.4±0.2
Camelina sativa	15.2± 3.8	2.8±0.2

[1] Feeding Level = $\sum$ (no. of small feeding holes*1) + (no. of medium sized feeding holes*2) + (no. of large sized feeding holes*3).

Figure 1. Crucifer flea beetle feeding bioassay testing *Crambe abyssinica* leaf extracts dissolved in five solvents and placed on *B. napus* AC Excel leaf disks. Feeding deterrence index (± standard error of the mean) was calculated by subtracting average leaf area eaten on disks with *C. abyssinica* leaf extracts plus solvent from control feeding levels (leaf disks treated with solvent alone), dividing by leaf area eaten on both types, and multiplying by 100%. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.



Solvent and Concentration of Extract Tested

Figure 2. Leaf disk bioassay determining feeding deterrence index (±SEM) of *Crambe abyssinica* leaf extracts tested against flea beetles in the laboratory, September, 2000. Feeding deterrence index calculated by subtracting average leaf area eaten of *B. napus* cv AC Excel leaf disks upon which was eluted three concentrations of *C. abyssinica* leaf extracts dissolved in four solvents from control feeding levels (leaf disks with solvent alone), dividing by leaf area eaten on both types, and multiplying by 100%. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.

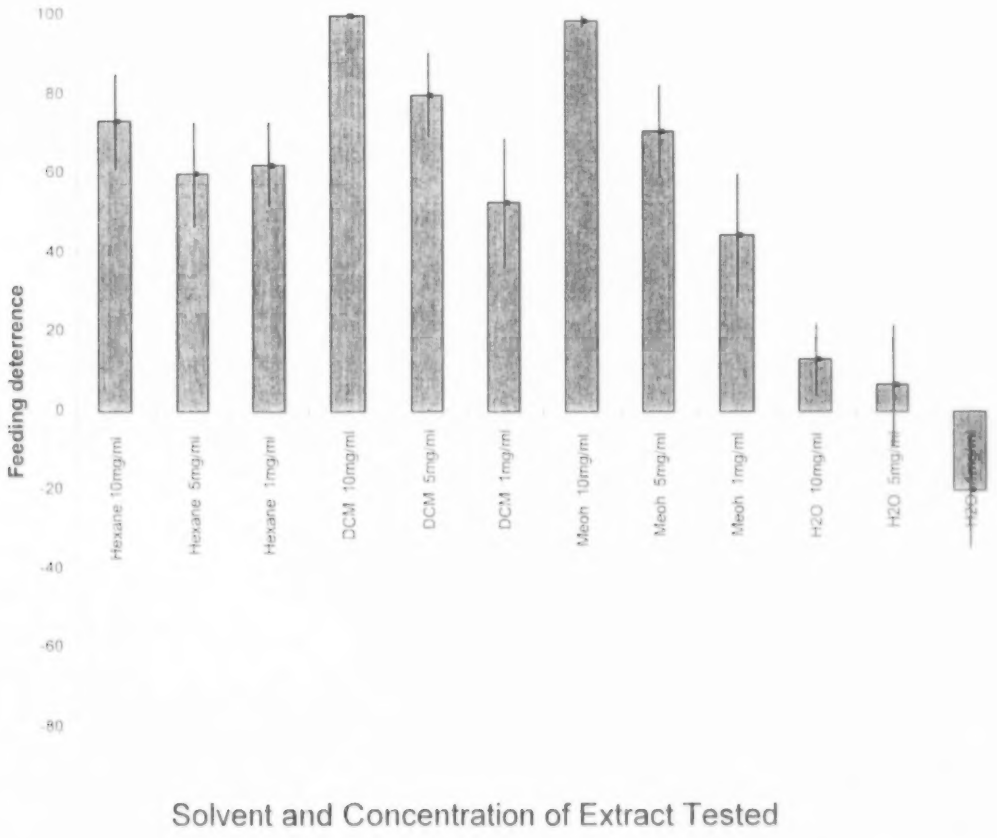
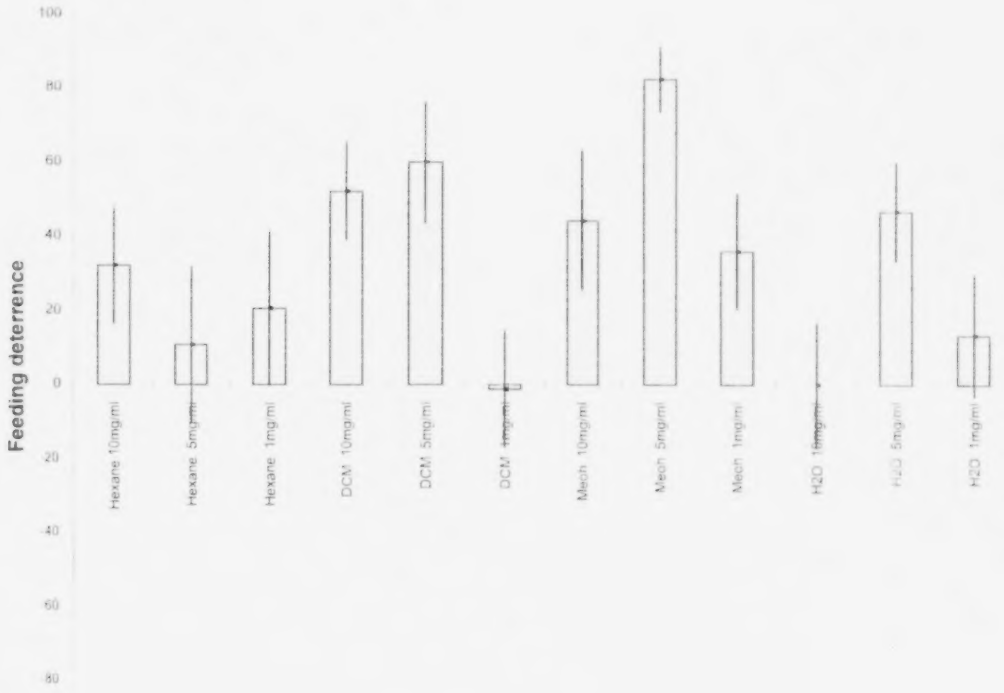


Figure 3. Leaf disk bioassay determining feeding deterrence index (±SEM) of *Thlaspi arvense* leaf extracts tested against flea beetles in the laboratory, September, 2000. Feeding deterrence index calculated by subtracting average leaf area eaten of *B. napus* cv AC Excel leaf disks upon which was eluted the concentrations of *T. arvense* leaf extracts dissolved in four solvents from control feeding levels (leaf disks with solvent alone), dividing by leaf area eaten on both types, and multiplying by 100%. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.



Solvent and Concentration of Extract Tested

Figure 4. Leaf disk bioassay determining feeding deterrence index (±SEM) of *Camelina sativa* leaf extracts tested against flea beetles in the laboratory, September, 2000. Feeding deterrence index calculated by subtracting average leaf area eaten of *B. napus* cv AC Excel leaf disks upon which was eluted three concentrations of *C. sativa* leaf extracts dissolved in five solvents from control feeding levels (leaf disks with solvent alone), dividing by leaf area eaten on both types, and multiplying by 100%. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.

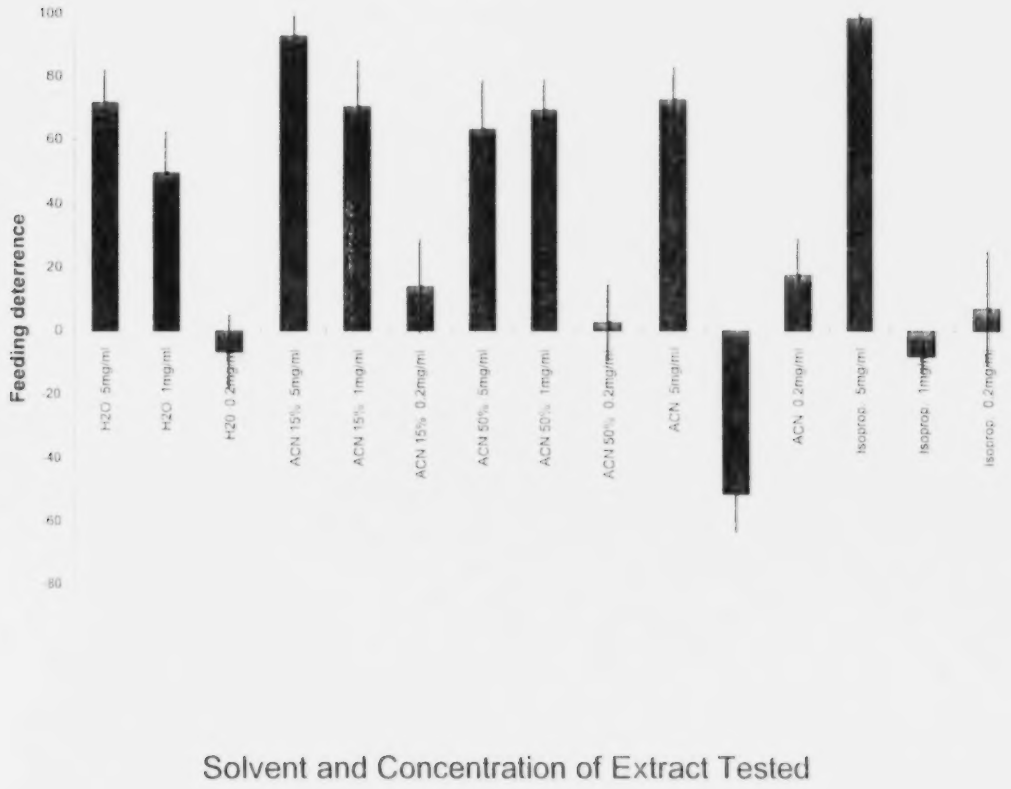


Figure 5. Leaf disk bioassay determining feeding deterrence index (±SEM) of three concentrations of *Camelina sativa* leaf extracts dissolved in three solvents and placed on leaf

disks of *B. napus* cv AC Excel tested against flea beetles in the laboratory. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.

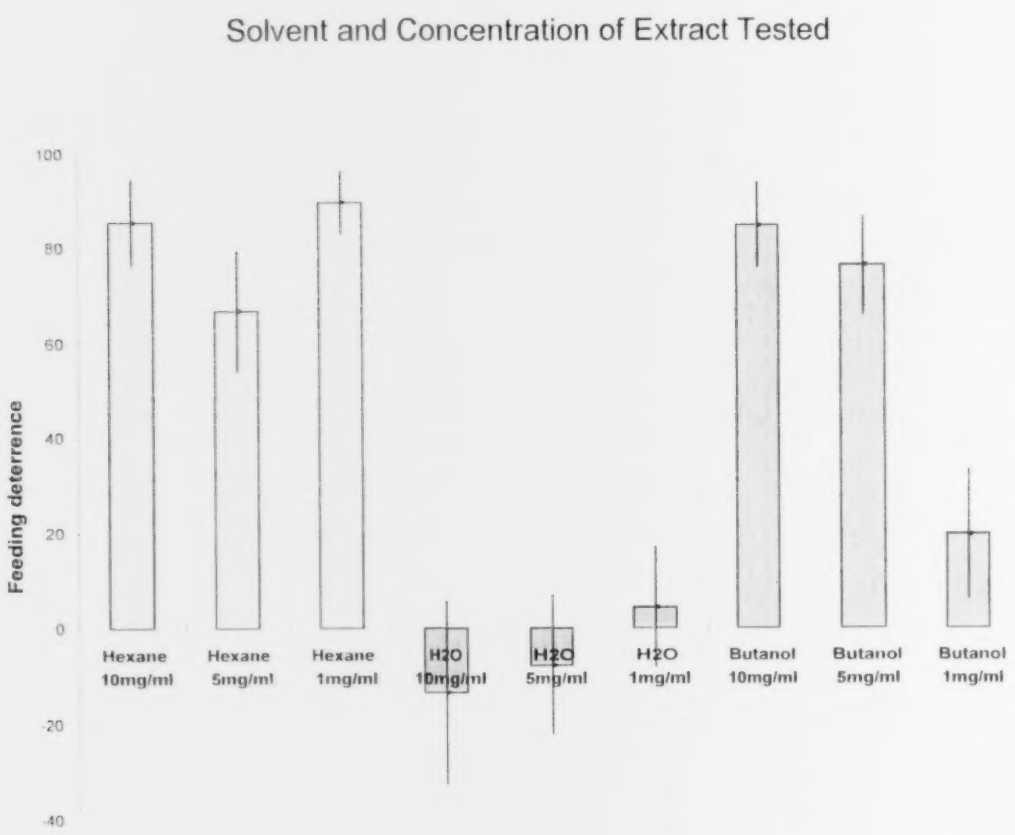


Figure 6. High pressure liquid chromatography fractions of *Crambe abyssinica* and *Thlaspi arvense* leaf extracts placed on disks of *Brassica napus* leaves and evaluated for flea beetle resistance. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.

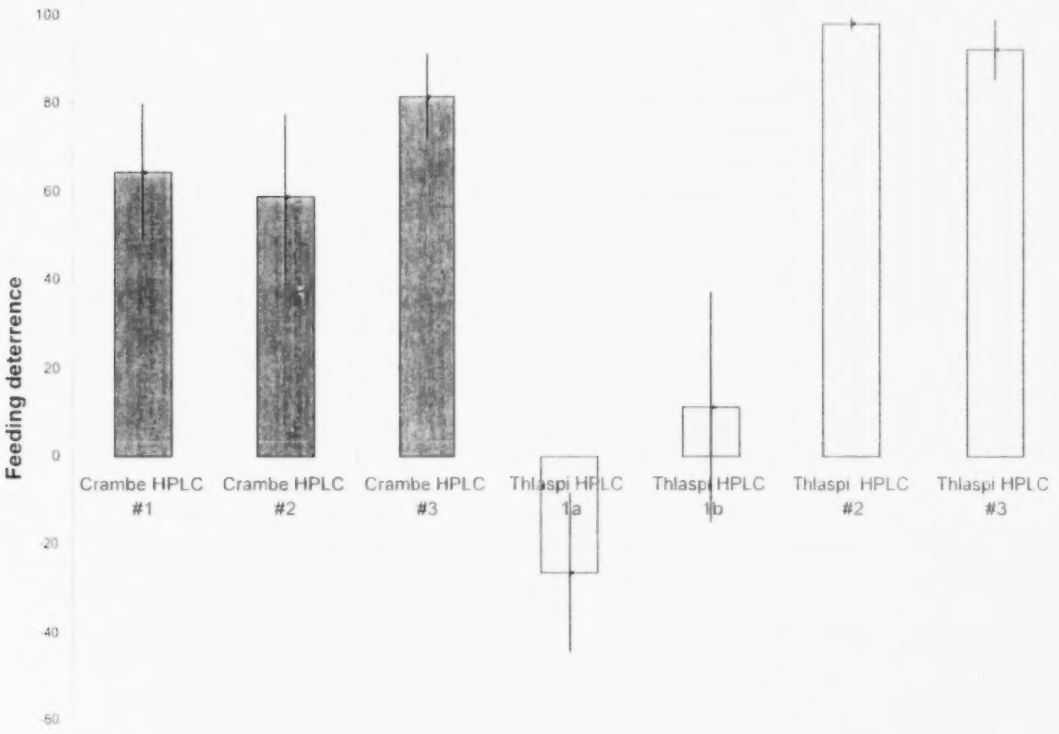


Figure 7. High pressure liquid chromatography fractions of *Camelina sativa* leaf extracts placed on disks of *Brassica napus* leaves and evaluated for flea beetle resistance. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.

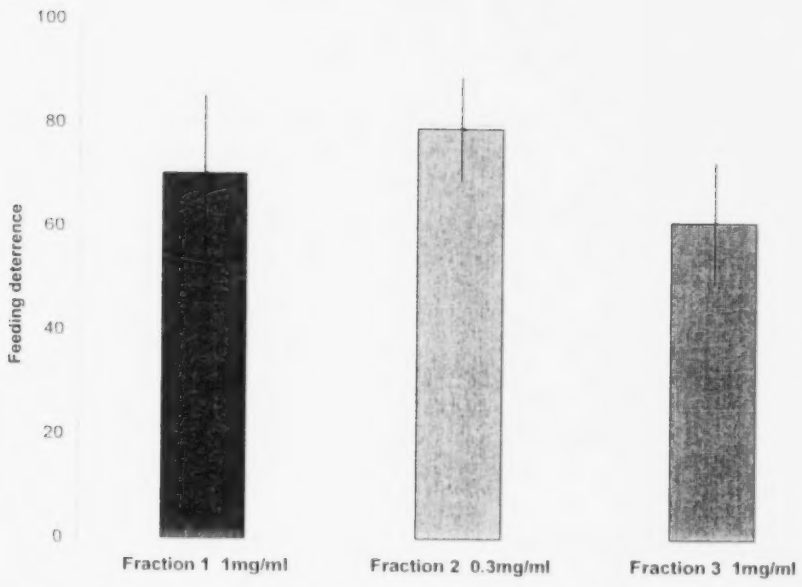


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Figure 8. Crucifer flea beetle feeding bioassay testing trichome-enhanced transgenic *Brassica napus* cv 'Westar' against untransformed 'Westar' 48 hours after feeding commenced. Feeding deterrence index (± standard error of the mean) was calculated by subtracting average leaf area eaten on mutant lines from feeding on *B. napus* cv. Westar controls, dividing by leaf area eaten on both types, and multiplying by 100%. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation.

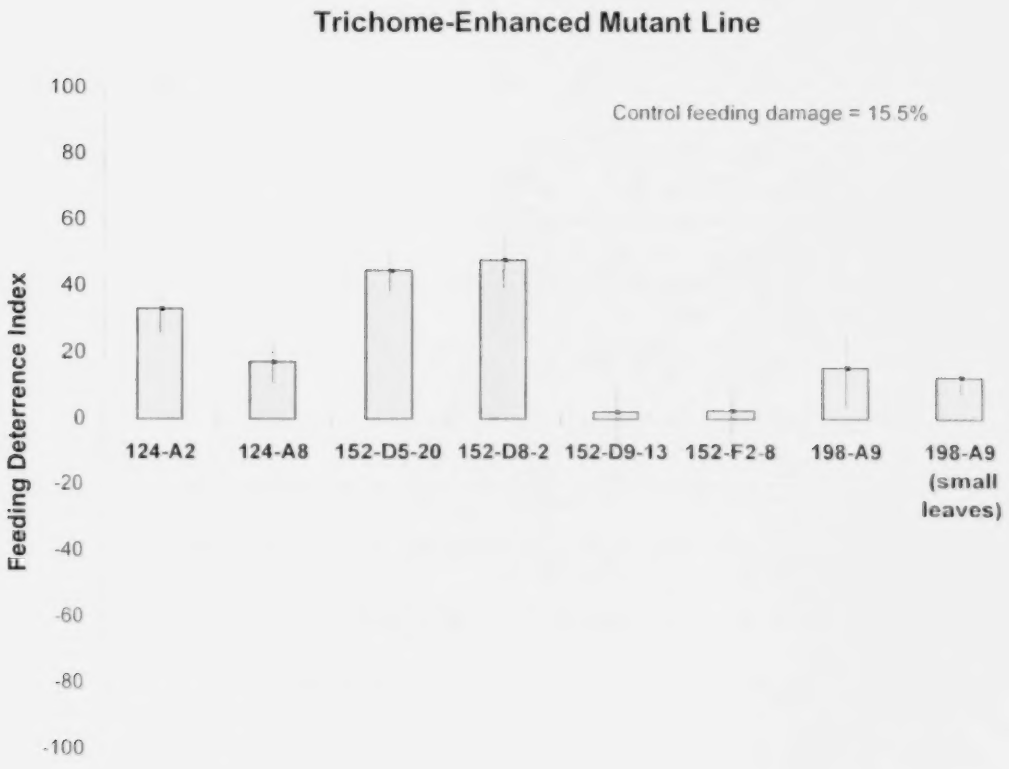


Figure 9. Percent cotyledon and first true leaf damage of *Arabidopsis* mutant plants eaten by flea beetles in field trials, Saskatoon, 2001.

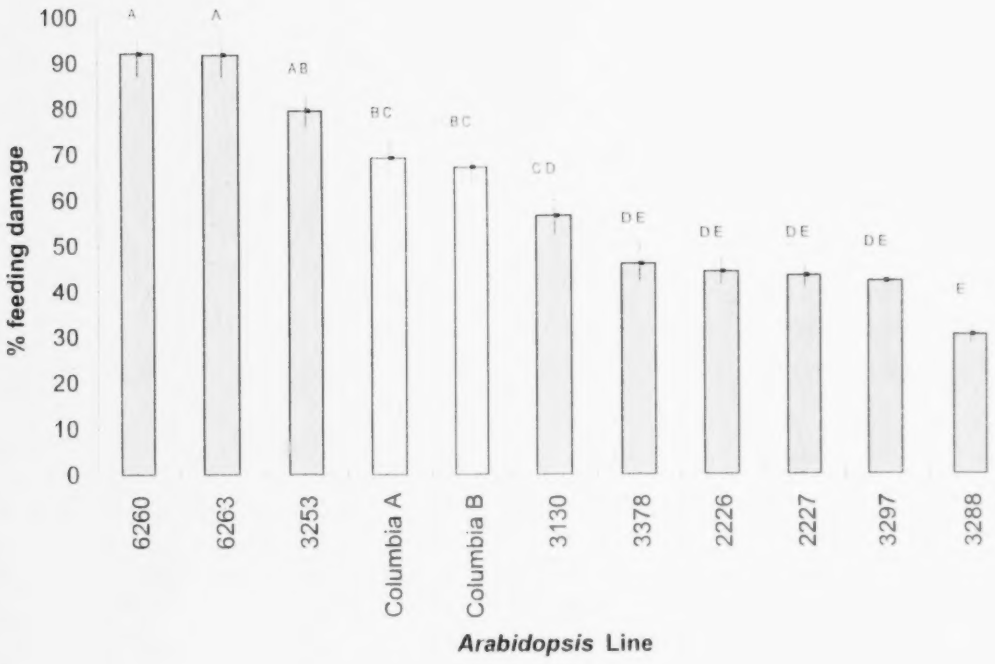


Figure 10. Numbers of summer generation flea beetles collected weekly from emergence cone traps set in plots of winter canola near Saskatoon 2000 and 2001.

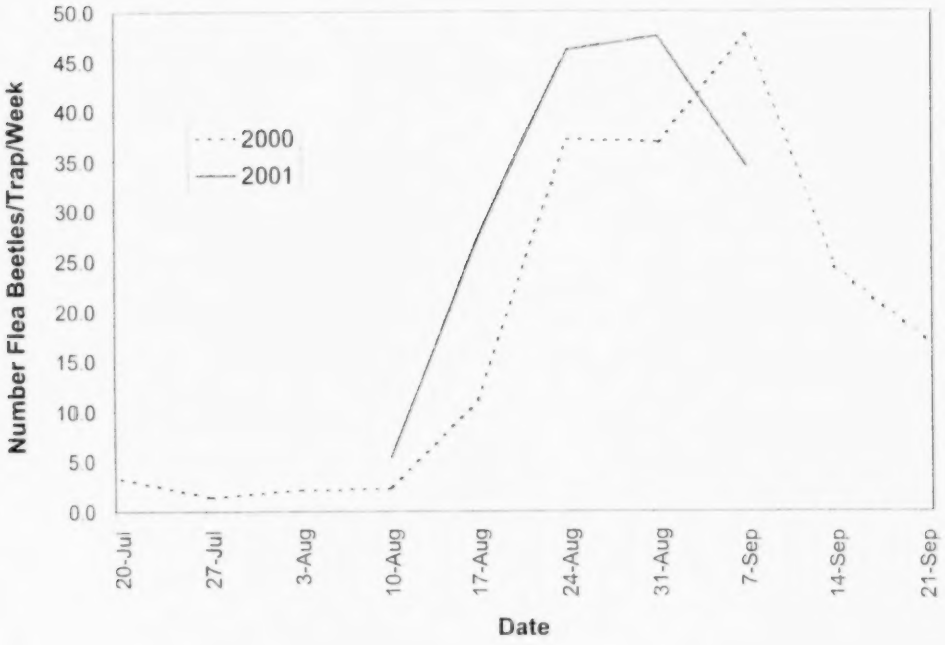


Figure 11. *B. napus* leaf disk bioassay determining feeding deterrence index (±SEM) of disks treated with flavone and flavone compounds against bertha armyworms in the laboratory. Positive values indicate feeding deterrence on test lines, negative values indicate feeding stimulation. Flavone compound 1 is 4-hydroxyflavone, 2 is 5-hydroxyflavone, 3 is 3,4-7-trihydroxyflavone, 4 is 5,7-dihydroxyflavone, 5 is luteolin, 6 is 7-hydroxyflavone, 7 is apigenin, and 8 is 7,4-dihydroyflavone.

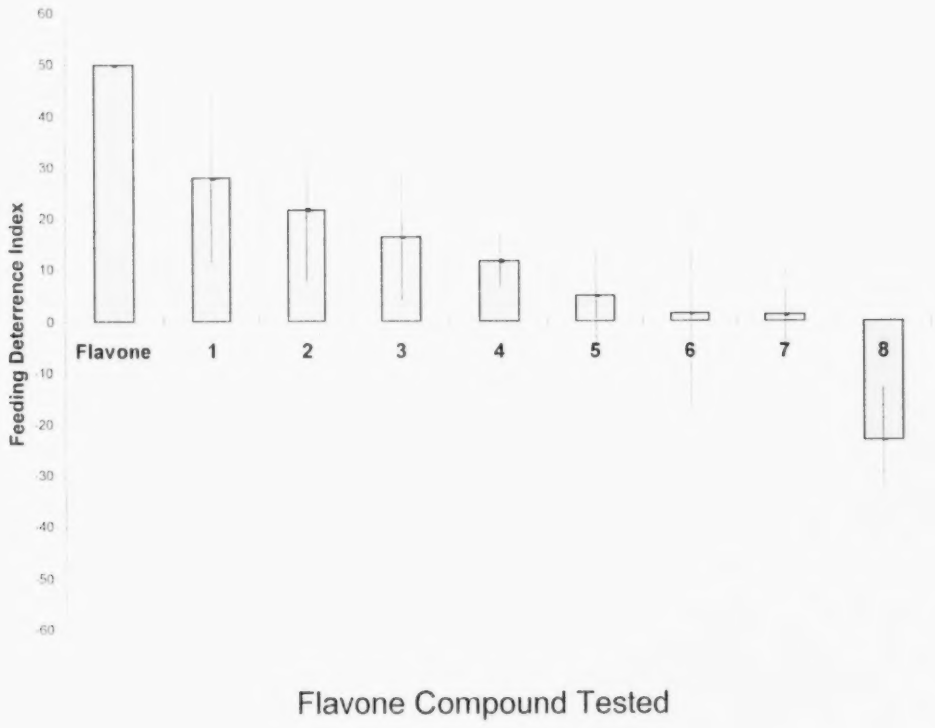


Figure 12. Bioassay measuring feeding deterrence of leaf disks of *Brassica napus* cv AC Excel treated with serial dilutions of flavone towards flea beetles in the laboratory. The greater the feeding deterrence index, the greater the repulsion of the compound to flea beetles.

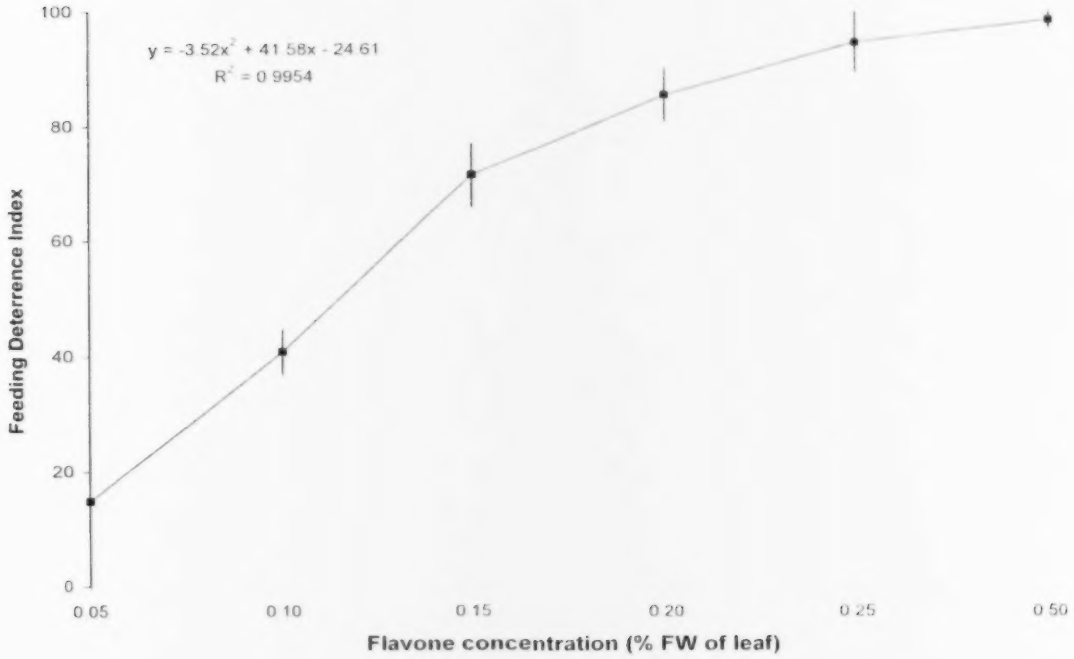
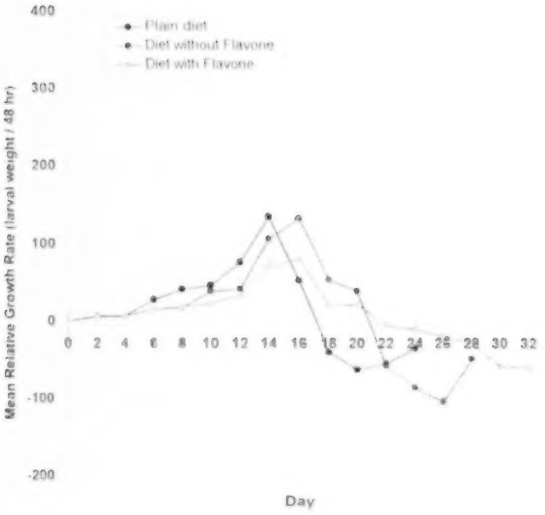


Figure 13. Effect of flavone at a) 150 g. and b) 200 g. per gram of diet on mean relative growth rate of bertha armyworm larvae, tested with a plain diet control and a diet with solvent only.

a)



b)

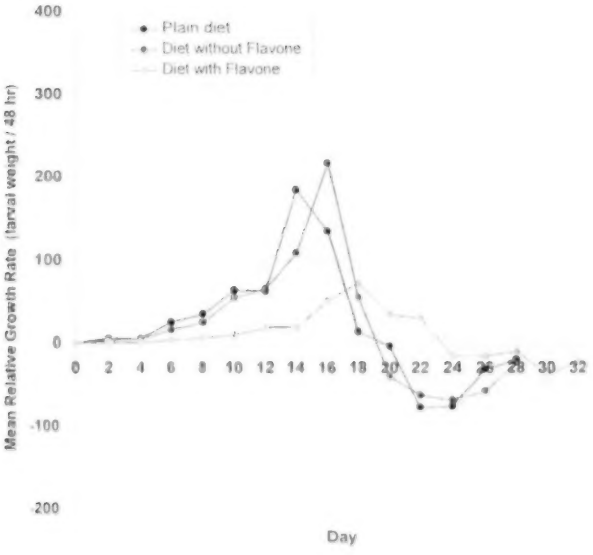
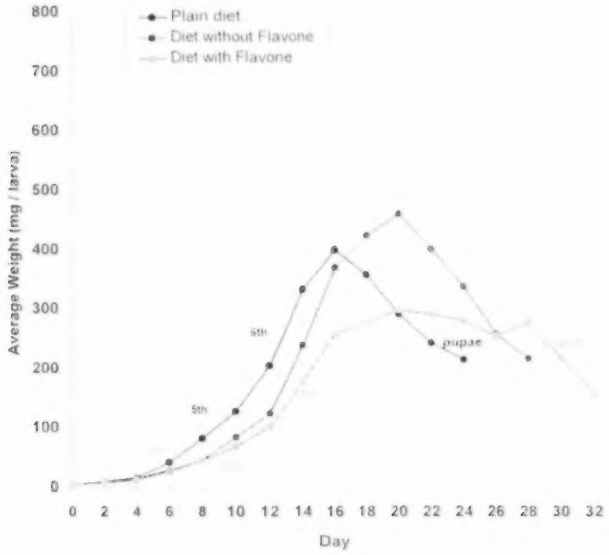
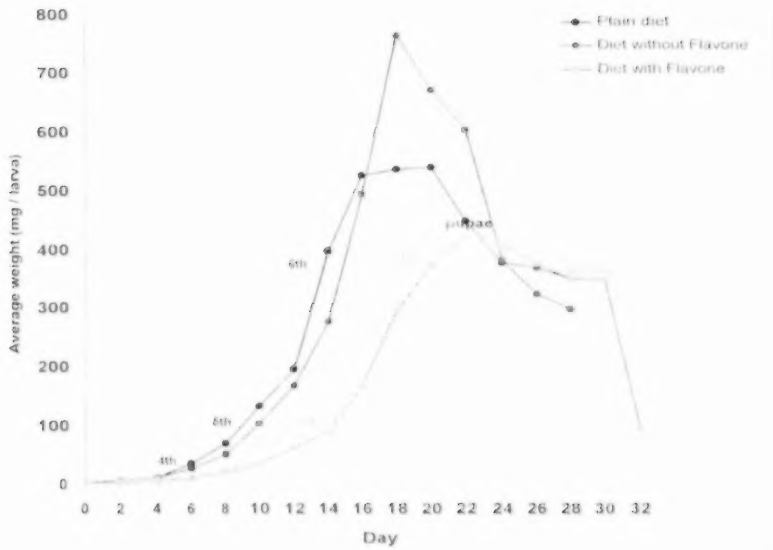


Figure 14. Effect of flavone at a) 150 g. and b) 200 g. per gram of diet of bertha armyworm on larval weight, tested with a plain diet control and a diet with solvent only. Insect growth stage indicated on growth line.

a)



b)



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APPENDIX

FLEA BEETLE - LEAF DISK BIOASSAY PROTOCOL

1. PREPARATION

Plants

Seeds were obtained from the seed bank of the Saskatoon Research Centre and the collection of J. Soroka. The control used was *Brassica napus* cultivar AC Excel (JS99-336). Seeds were planted in 13 cm diameter pots containing Redi-Earth Soil-less Mix. Seeds germinated and were allowed to grow to the 4 – 5 week stage. Conditions in the greenhouse section E1 and E2 were 18℃ days and 16℃ nights with a 16 hour photoperiod.

Flea Beetles

The flea beetles used in bioassays were field collected from allyl isothiocyanate traps or by sweeping. The flea beetles were brought into the lab and stored in square plexiglass / screen cages measuring 45cm on each side. A cotton sleeve on the front allowed for access into the cage. The flea beetles were held for a minimum of 24 hours before being used in a bioassay. During the 24 hour pre-bioassay period the beetles were provided with a water source only so as to starve the beetles so they would be more likely to feed during the bioassay. Aside from the pre-bioassay period the beetles were fed commercially obtained cabbage: distilled water was periodically sprayed on the mesh sides of the cages. The conditions of the flea beetle maintenance room (SB5.1) were a 21℃ day, 19℃ night and a 16 hour photoperiod.

Agar plates

A 1% solution of Difco agar (Fisher Scientific Co.) was autoclaved for 15 minutes in distilled water. Fertilizer (20-20-20) at a concentration of 1.7g / L. was added to the agar before autoclaving to aid in keeping the leaf disks alive. This agar solution was used in experiment plates described below or glass petri-dishes (9cm) to keep the disks moist between extract applications. Filter paper was put on top of all agar plates.

The experimental agar plates were poured the day before or on the morning of bioassay set-up. The 60 x 15 mm disposable plastic petri plates (Fisher Scientific Co.) were labelled on the lid to indicate which extract the plate contained. After each bioassay the plates were emptied, washed with detergent, rinsed thoroughly and then reused. Agar plates more than 1 week old were not used in any bioassay due to decreasing media quality.

2. BIOASSAY

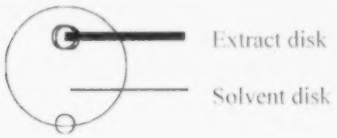
Leaf Disks

Leaf disks were cut from *B. napus* plants at the 4 –5 week growth stage with a #5 cork borer (disk area approx. 80 mm^2). Replicates for a trial used leaf disks from the same leaf. Disks were cut between and not on leaf veins. Disks were not cut less than 0.5 cm from the margin of the leaf. Only disks that were flat and had no nicks or tears along the margins were used. Leaf disks were either transferred to a glass petri-dish for immediate application of the extract or else they were put on large (9cm) agar plates with filter paper.

Extract application

If an extract was in a solvent that was easily spread over the surface, 10 microliters were applied topically to the leaf disk using a 20 microliter variable volume Gilson Pipettman.

If the extract was water-based and did not spread well, 10 µl of 0.05% Sillwet solution were applied prior to applying the extract. The extract was applied before the Sillwet dried. Once disks were dry they were transferred to the agar plate as shown below. Control plates (minimum 5 replicates) were also set up in order to see if flea beetle feeding or lack thereof was because of the extract treatment.



Adding Beetles

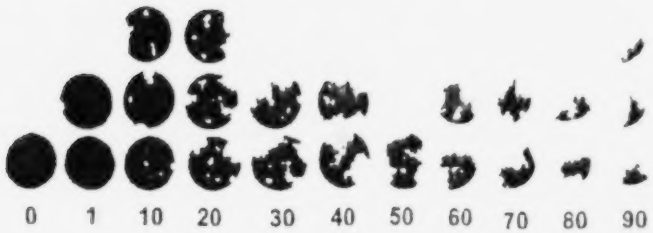
Flea beetles that were actively crawling on the sides of the cage were removed from the cage by means of vacuum suction. It is assumed that active beetles are healthy beetles. The beetles were then put in a glass vial on ice to slow them down during the transfer to the bioassay plates. The moisture on the lids was wiped off. Ten beetles were added to each plate and the plates were secured shut with an elastic band. The elastic band also seemed to keep moisture from forming on the inside of the lids during the bioassay. The plates were placed on the bench under high intensity fluorescent lights (10 lux).

Duration of bioassay

The bioassay was allowed to run for 24 hours and then rated according to a visual scale that measured feeding levels in percentage leaf (or disk) area eaten in increments of 10% (Photograph below). If there was little or no feeding on the controls, the bioassay was run for another 24 hours (total of 48 hours). However, this rarely happened. The ratings were entered directly on a palmtop computer and then transferred to a mainframe computer and analyzed.

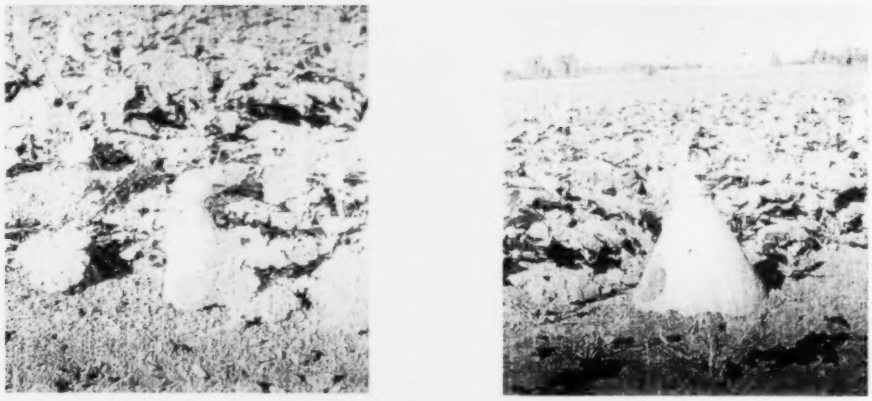
The standard rating scale for damage assessment of leaf disks is pictorially represented below:

Flea beetle leaf disk damage scale

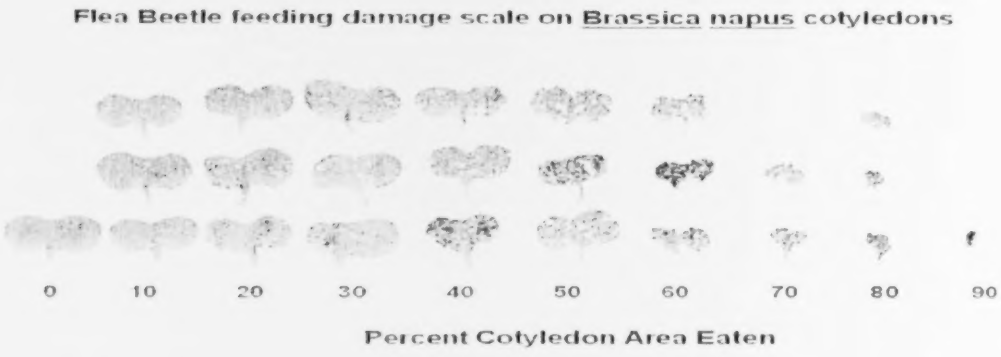


Percent Leaf Area Eaten

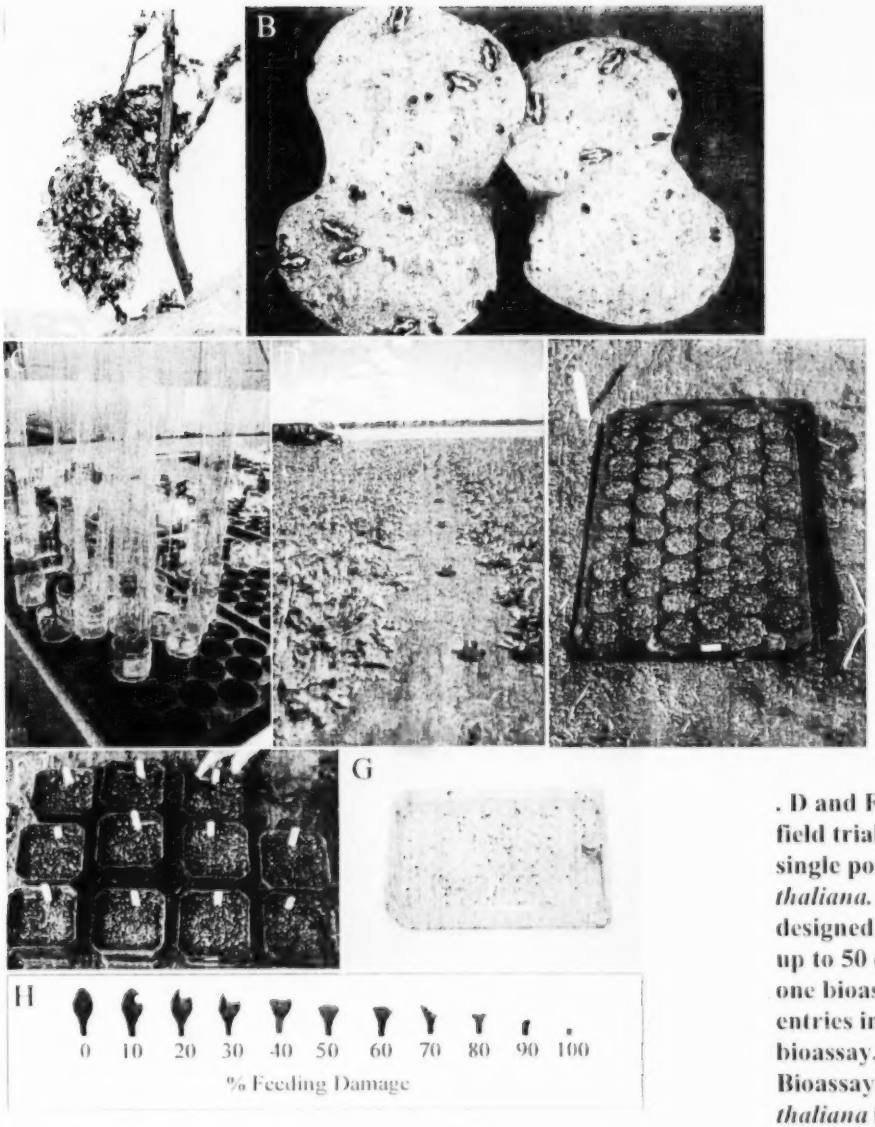
Project Photographs



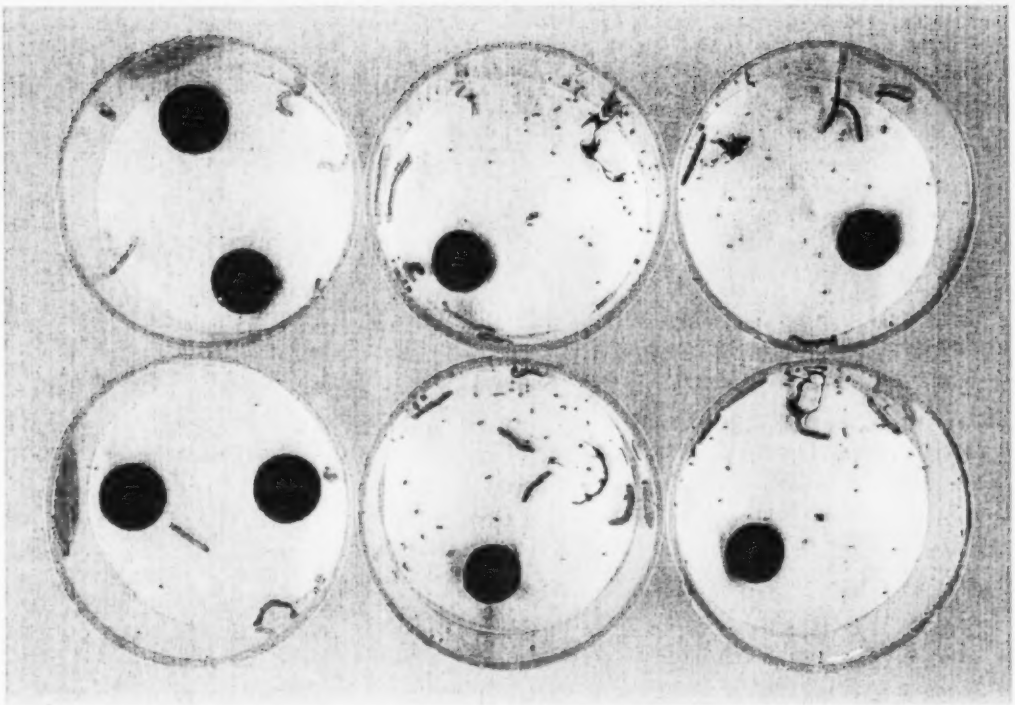
Photograph 1. Flea beetle collection traps. Left: Modified boll weevil trap baited with allyl isothiocyanate at the top of the yellow cone. Flea beetles enter the trap between the yellow and tan plastic segments. Right: Emergence cone trap. This trap collects flea beetles emerging from pupation in the soil.



Photograph 2. Scale of flea beetle feeding damage to *Brassica napus* cotyledons as assessed by both a human observer and by a digital imaging system.



Photograph 3 A and B. Flea beetle feeding. C. Growing ***Arabidopsis thaliana*** **in the laboratory. D and F. Initial field trial using single pots of *A. thaliana*. E. System designed to assess up to 50 entries in one bioassay entries in a field bioassay. G. Bioassaying *A. thaliana* in the laboratory. H. Feeding damage rating scale of *A. thaliana* cotyledons.**



Photograph 4. Bertha armyworm leaf disk choice bioassay. Left arenas - test initiation; middle and right arenas - after 18 hours of feeding on highly susceptible vs resistant treatment.

ADDENDUM: Responses of Crucifer Insects to Commercial Phytochemicals (Feeding Deterrent Indices unless Otherwise Indicated)

FB - Flea Beetle Phyllotreta cruciferae
BAW - Bertha Armyworm Mamestra configurata
DBM - Diamondback Moth Plutella xylostella

N = not tested

	10mg/ml	10mg/ml	4mg/ml	10mg/ml	4mg/ml
Coumarin derivatives	**FB**	**BAW**	**BAW**	**DBM**	**DBM**
Coumarin	60	90	12	97	74
3-Hydroxycoumarin	N	44	36	65	47
4-Ethoxycoumarin	91	96	37	89	80
4-Hydroxy-6-methylcoumarin	70	61	17	54	48
4-Hydroxycoumarin	95	20	24	52	40
4-Methoxycoumarin	95	69	84	76	64
4-Methyl Esculetin	60	18	7	35	45
4-Methyl-7-hydroxycoumarin	40	33	25	63	26
5,7-Dimethoxycoumarin	68	65	61	45	24

6,7-Dimethoxy-4-methylcoumarin		N	59	46	38	52
6,7-Dimethoxycoumarin		62	86	41	58	33
6-Hydroxy-7-methoxycoumarin		81	11	4	13	-4
7- Methoxycoumarin		94	86	49	69	49
	5mg/ml	N	79	N	52	N
	3mg/ml	N	61	N	2	N
	2mg/ml	N	59	N	58	N
	1mg/ml	N	66	N	43	N
7-amino-4-methylcoumarin		N	25	51	46	32
7-Diethylamino-4-methylcoumarin		79	82	83	87	74
7-Dimethylamino-4-methylcoumarin		82	51	41	78	72
7-Ethoxy-4-methylcoumarin		85	74	41	78	90
7-Ethoxycoumarin		75	90	45	82	67
7-Methylcoumarin		95	89	6	64	64
Esculetin (6,7-Dihydroxycoumarin)		0	34	18	68	-5
Scopoletin (6-Methoxy-7-hydroxycoumarin)		33	48	23	72	9
Umbelliferone (7-Hydroxycoumarin)		7	64	39	12	31

Flavonoids and isoflavonoids	**2.25mM FB**	**2.25mM BAW**	**10mg/ml DBM**
Flavone	77	N	93
2,3-Dihydroflavone	N	41	N
3',4',7-Trihydroxyflavone	N	16	N
4'-Hydroxyflavone	N	29	N
5,7 Dihydroxyflavone	N	N	N
5-Deoxykaempferol	N	18	N
5-Hydroxyflavone	N	20	N
5-Methoxyflavanone	52	1	N
6-Hydroxyflavanone	4	-1	N
7,4'-Dihydroxyflavone	-9	-23	N
7-Hydroxyflavone	N	2	N
Apigenin	-31	1	N
Chrysin	N	12	N
Deidzein	-9	N	N
Dihydrokaempferol	16	3	N
Dihydroquercetin	44	-29	N
Eriodictyol	20	19	N
Fisetin	N	6	N
Flavonol (3-Hydroxyflavone)	N	-8	N
Hesperetin	-5	-13	N
Hesperidine	N	8	N
Isorhamnetin	-12	24	N
Isorhamnetin-3-sophoroside-7-glucoside	N	45	N
Kaempferol	-10	3	N
Kaempferol-3,7-diglucoside	N	21	N
Kaempferol-3-glucoside	N	18	N
Kaempferol-3-rhamnoside	N	27	N
Kaempferol-3-rutinoside	N	13	N
Kaempferol-3-sophoroside-7-diglucoside	N	-3	N
Kaempferol-4-O-methyl ether	N	22	N
Liquiritigenin	-16	N	N

Luteolin	13	5	N
Myricetin	N	32	N
Myricetin-3-rhamnoside	N	-7	N
Naringenin	N	-15	N
Naringenin-chalcone	N	N	N
Naringin	-7	36	N
Phloretin	2	N	N
Quercetin	4	-8	N
Quercetin-3 -rhamnoside	N	35	N
Quercetin-3-glucoside	N	12	N
Rutin	see below	-5	N

Flea Beetles

	Rutin FDI	Quercetin FDI	Kaempferol FDI
10mg/ml	5	-30	-5
5mg/ml	43	16	9
4mg/ml	0	-31	-16
3mg/ml	27	-11	-36
2mg/ml	17	36	-48
1mg/ml	40	-40	-21

Phenolics		**10mg/ml FB**	**10mg/ml BAW**	**10mg/ml DBM**
2,6 Dihydroxybenzoic Acid (gamma-resorcyclic)		N	59	99
2-Hydroxybenzoic Acid (salicyclic)		N	N	N
4-Hydroxybenzoic Acid				
Caffeic Acid	*10mg/ml*	N	11	30
	5mg/ml	100	N	N
	3mg/ml	95	N	N
	1mg/ml	94	N	N
	0.5mg/ml	35	N	N
Chlorogenic Acid	*10mg/ml*	N	3	12
	5mg/ml	99	12	N
	3mg/ml	82	21	N
	1mg/ml	59	25	N
	0.5mg/ml	89	-23	N
4-hydroxy-3-methoxycinnamic Acid (ferulic)		N	32	26
2-hydroxycinnamic (o-coumaric acid)		Y	N	N
4-hydroxycinnamic acid (p-coumaric acid)		Y	N	N
Trans-cinnamic Acid		N	N	N

Phenolic polymers		**FB**	***Etoh* 10mg/ml BAW**	***H2O/Meoh* 10mg/ml BAW**	**10mg/ml DBM**
Lignan	*20mg/ml*	86	N	N	N
	10mg/ml	52	-23	N	N
	5mg/ml	66	N	N	N
	1mg/ml	80	N	N	N
Mixed Tannin		N	-17	23	19
Tannin 095		N	8	7	24

Tannin 193		N	6	33	36
Tannin 196		N	9	30	26
Tannin 229		N	8	47	46
Tannin 265		N	18	28	45

		20mg/ml	10mg/ml	
Terpenes and other volatiles		**FB**	**BAW**	**DBM**
B-Ionone		72	Y	Y
(+)-Alpha-pinene		N	4	N
Eucalyptol (1,8-cineole)		N	57	N
(+)-limonene		N	-18	N
Farnesene		N	90	N
(-)-Trans-caryophyllene		N	79	N
(-)-Alpha-cednene		N	41	N
Myrcene		N	-13	N
(+)-sabinene		N	-15	N
Ocimene		N	-7	N
B-Pinene		N	19	N

Cardenolides		**FB**	**BAW**	**DBM**
Digitoxin	*3mg/ml*	100	N	N
	1mg/ml	84	N	N
	0.5mg/ml	100	N	N

Indoles		**FB**	**BAW**	**DBM**
Indole	*10mg/ml*	Y	10	N
	5mg/ml	N	100	N
	2.5mg/ml	N	22	N